O.I.P.E. 14:50
APR 28 2006
PATENT & TRADEMARK OFFICE

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MO: MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSL

HU: GGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFTGTAG
MO: GGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTLHFSGQFTGTAG

HU: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS
MO: ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS

HU: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG
MO: YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG

HU: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTE
MO: SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTE

HU: GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS
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HU: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR
MO: ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR

HU: SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR
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Fig. 1

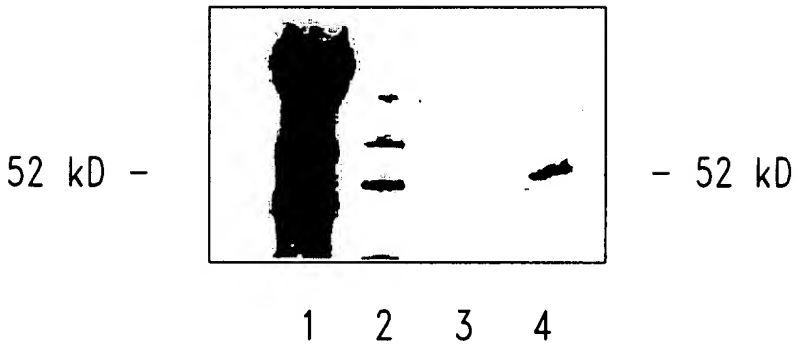


Fig. 2

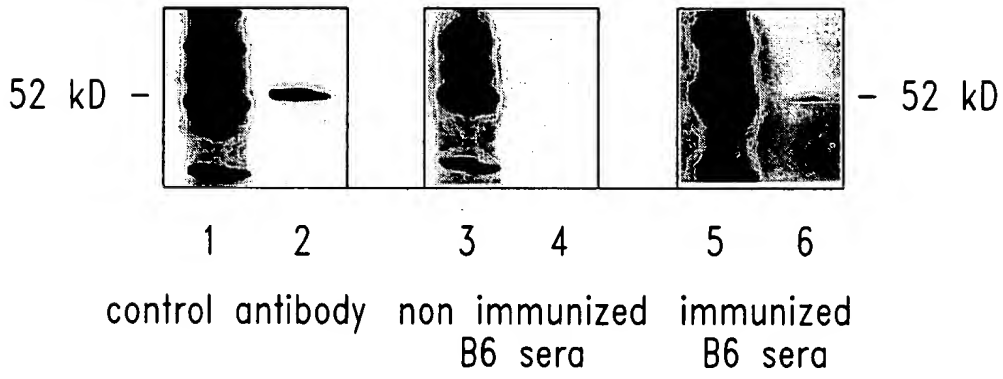

52 kD
52 kD
1 2 3 4 5 6
control antibody
non immunized B6 sera
immunized B6 sera

Fig. 3

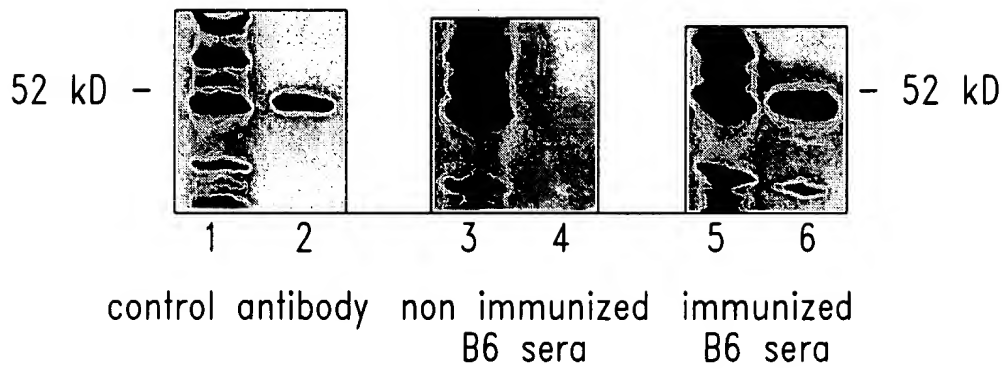

52 kD
52 kD
1
2
3
4
5
6
control antibody
non immunized B6 sera
immunized B6 sera

Fig. 4

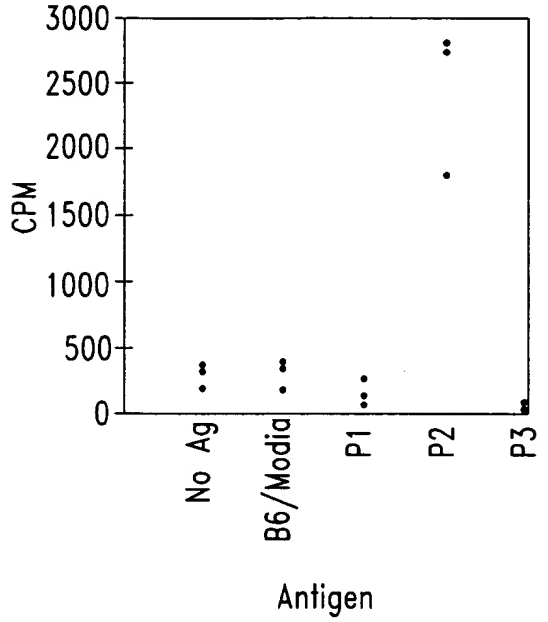


Fig. 5A

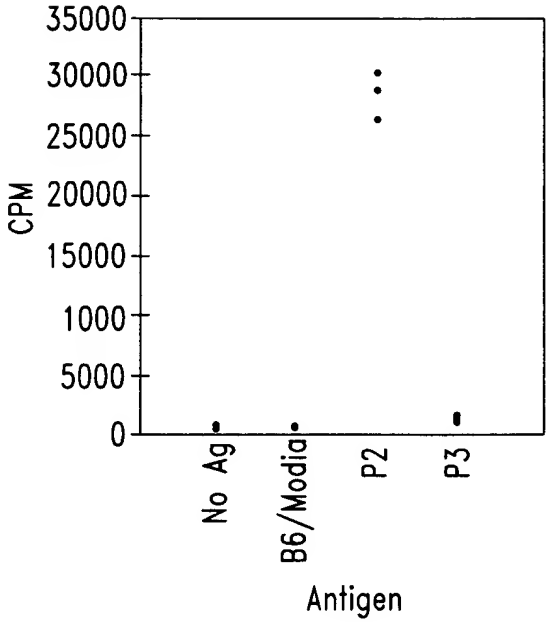


Fig. 5B

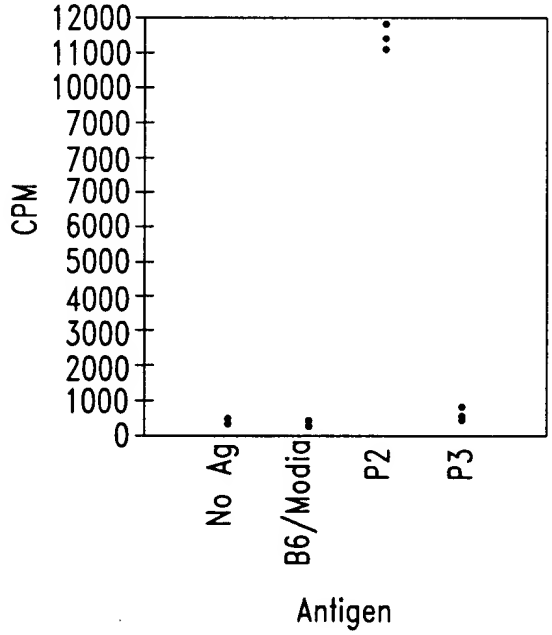


Fig. 5C

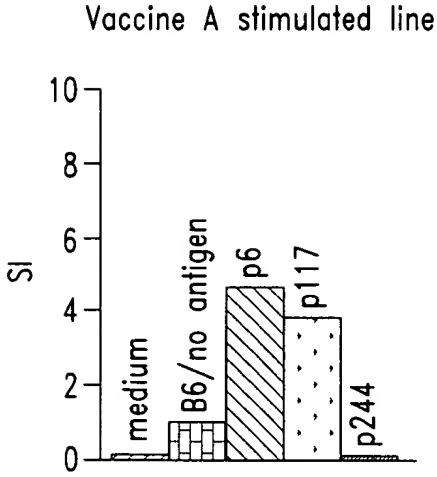
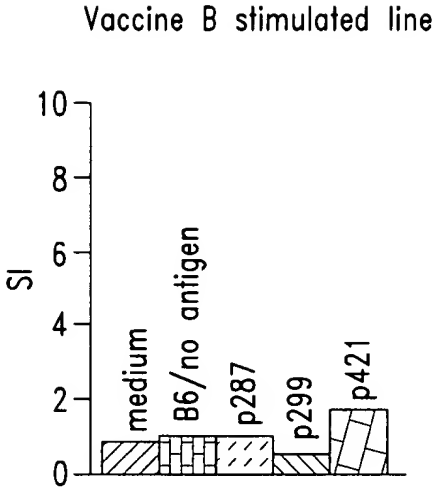

Vaccine A stimulated line
SI
10
8
6
4
2
0
medium
B6/no antigen
p6
p117
p244

Fig. 6A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SI
10
8
6
4
2
0
medium
B6/no antigen
p287
p299
p421

Fig. 6B

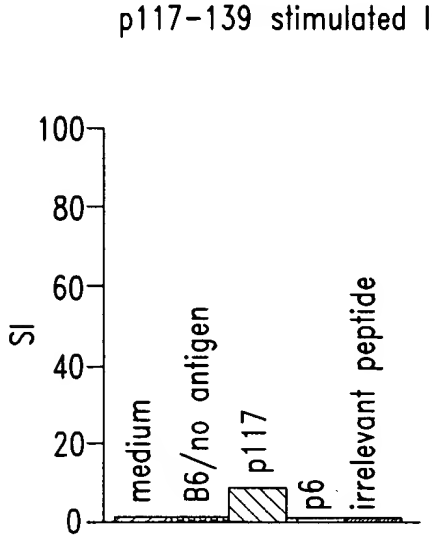

Fig. 7A

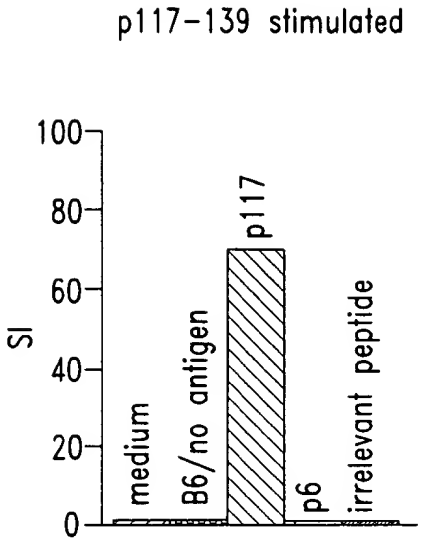

Fig. 7B

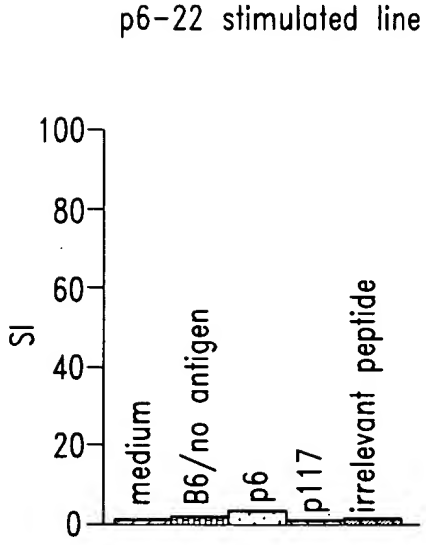

Fig. 7C

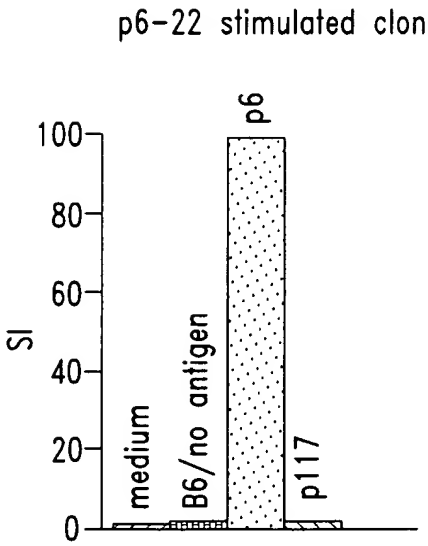

Fig. 7D

```
    5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQE
.....AAAAAAAAAAAAAAAA.......AAAAAA.........AAAAAAAAAAAA.....................
.............................RRRR.....................................
.....................................................................
.....................................................................

   80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
PSWGGAEPHEEQCLSAFTVHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYS
..............AAA......AAAA.....................AAA......AAAAAA............
.........................RRRR.....................RRRRR.....................
.....................................DDDDDDDDD.....................
.....................................................................

  155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGTPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
..............AAAAA.............................AAAAAA..............AA
..............RRRR.....................................................
.....................................................DDDDDDDDDDDDD...
.....................................................................

  230  235  240  245  250  255  260  265  270  275  280  285  290  295  300
LYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTEGQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDV
AAAAAAAA............AAA.AAA.....................................AAAAAAAAAAA
................RRRRRRRRR.....RRRR.........................RRRR.....
DDDDDD.............DDDDDDDDDD.....................................
.....................................................ddddd.........

  305  310  315  320  325  330  335  340  345  350  355  360  365  370  375
RRVPGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
AAAAA..AAAAAAAAAA.....................................AAAA.AAAAAAAAA.
....RRRRR.....................RRRR.....................................
.......DDDDDD.....................................................
.....................................................................

  380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
.............AAAA.AAAA..AA.....AAAA..........AAA....AAAAAAAA...AAA........
.....................................................RRRR..RRRR.............
.....................................................................
.................ddddddddddddd.....................................
```

Fig. 8A

```
    5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPHSFIKQE
.....AAAAAAAAAAAAAAAA.......AAAAAA.........AAAAAAAAAAAA.....................
.............................RRRR.....................................
.....................................................................
.....................................................................

   80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
PSWGGAEPHEEQCLSAFTLHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYS
.........................AAAA.....................AAA......AAAAAA............
.........................RRRR.....................RRRRR.....................
.....................................DDDDDDDDD.....................
.....................................................................

  155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGAPSYGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
.................AAAAA.............................AAAAAA................AA
.................RRRR.....................................................
.....................................................DDDDDDDDDDDDD...
.....................................................................

  230  235  240  245  250  255  260  265  270  275  280  285  290  295  300
LYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTEGQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDV
AAAAAAAA.............AAA.AAA.....................................AAAAAAAAAAA
.................RRRRRRRRR.....RRRR.............................RRRR.....
DDDDDD..............DDDDDDDDDD.....................................
.....................................................ddddd.........

  305  310  315  320  325  330  335  340  345  350  355  360  365  370  375
RRVSGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
AAAAA..AAAAAAAAAA.....................................AAAA.AAAAAAAAA.
....RRRRR.....................RRRR.....................................
..DDDDDDDDDD.....................................................
.....................................................................

  380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWHSCQKKFARSDELVRHHNMHQRNMTKLHVAL
.............AAAA.AAAA..AA.....AAAA...........AA....AAAAAAAA...AAAA.......
.............................................RRRR..RRRR.................
.....................................................................
.................ddddddddddd.....................................
```

Fig. 8B

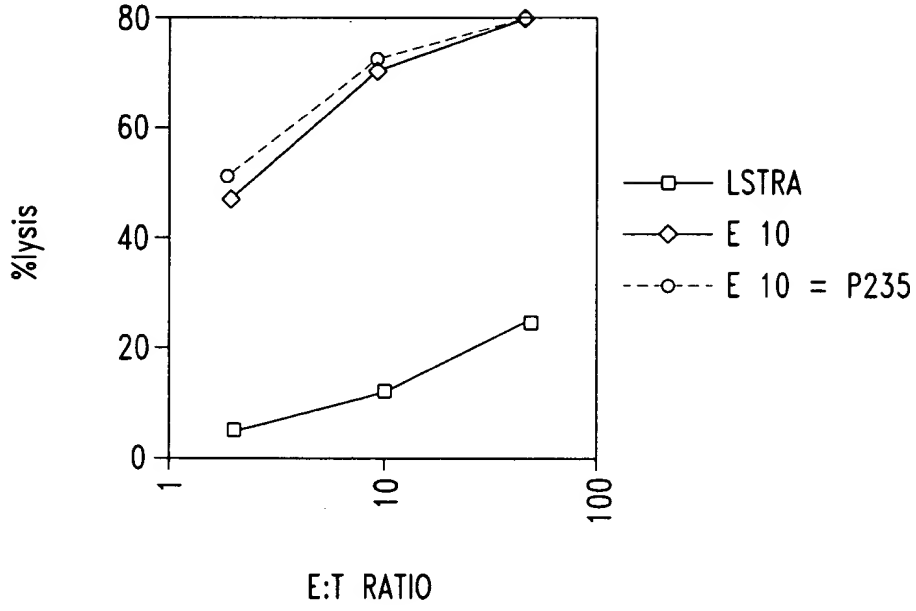
80
60
40
20
0
%lysis
1
10
100
E:T RATIO
LSTRA
E 10
E 10 = P235

Fig. 9A

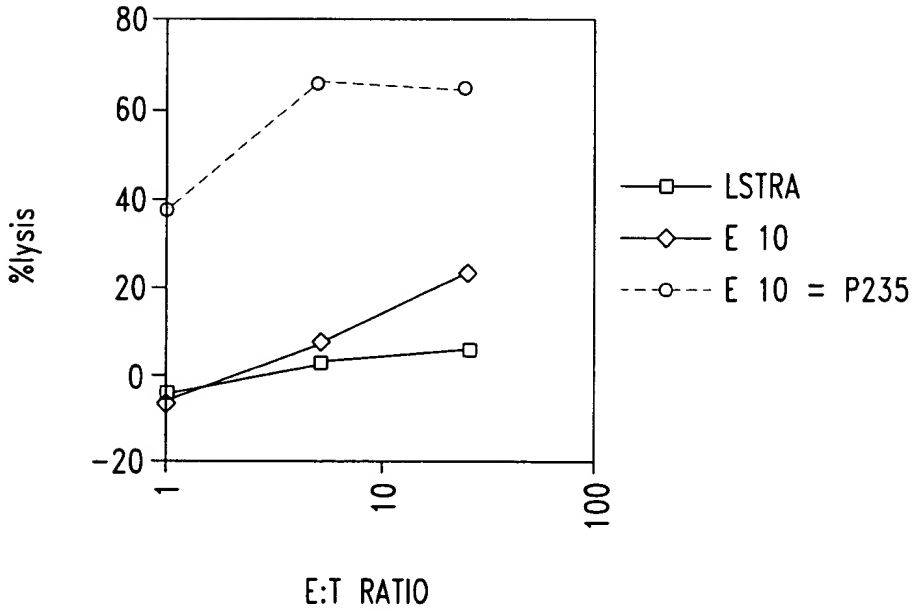
80
60
40
20
0
-20
%lysis
1
10
100
E:T RATIO
LSTRA
E 10
E 10 = P235

Fig. 9B

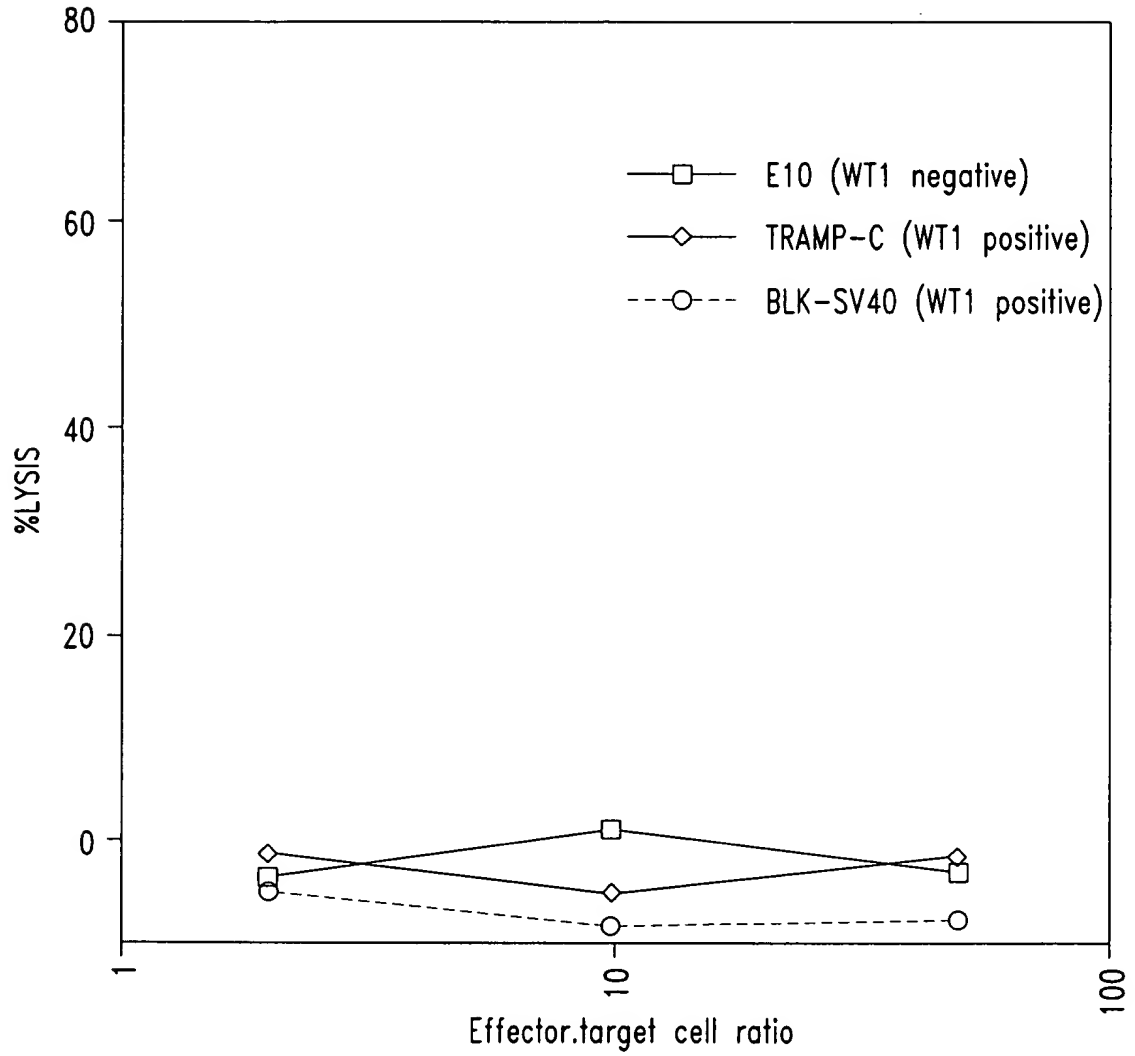
80
60
40
20
0
%LYSIS
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
1
10
100
Effector.target cell ratio

Fig. 10A

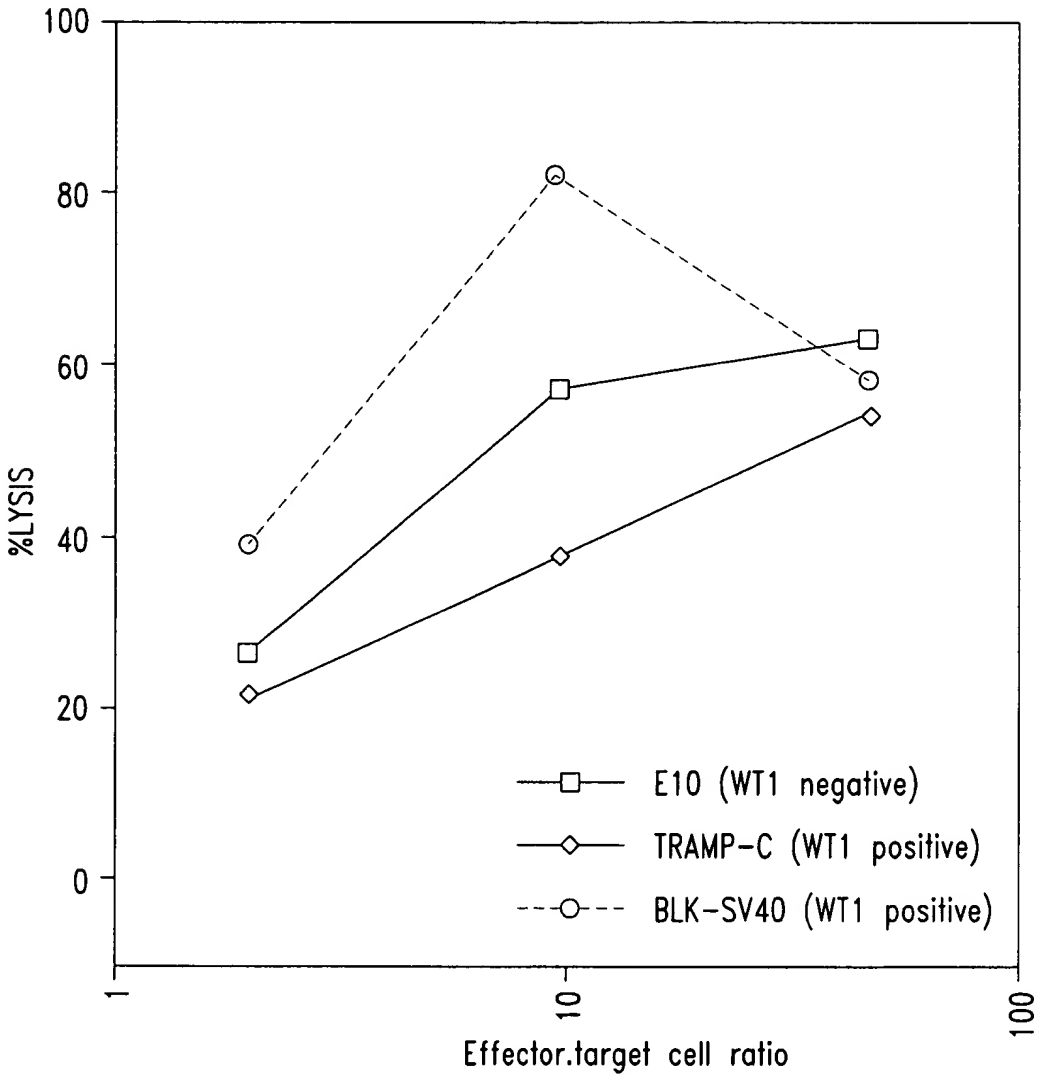
100
80
60
40
20
0
%LYSIS
1
10
100
Effector.target cell ratio
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)

Fig. 10B

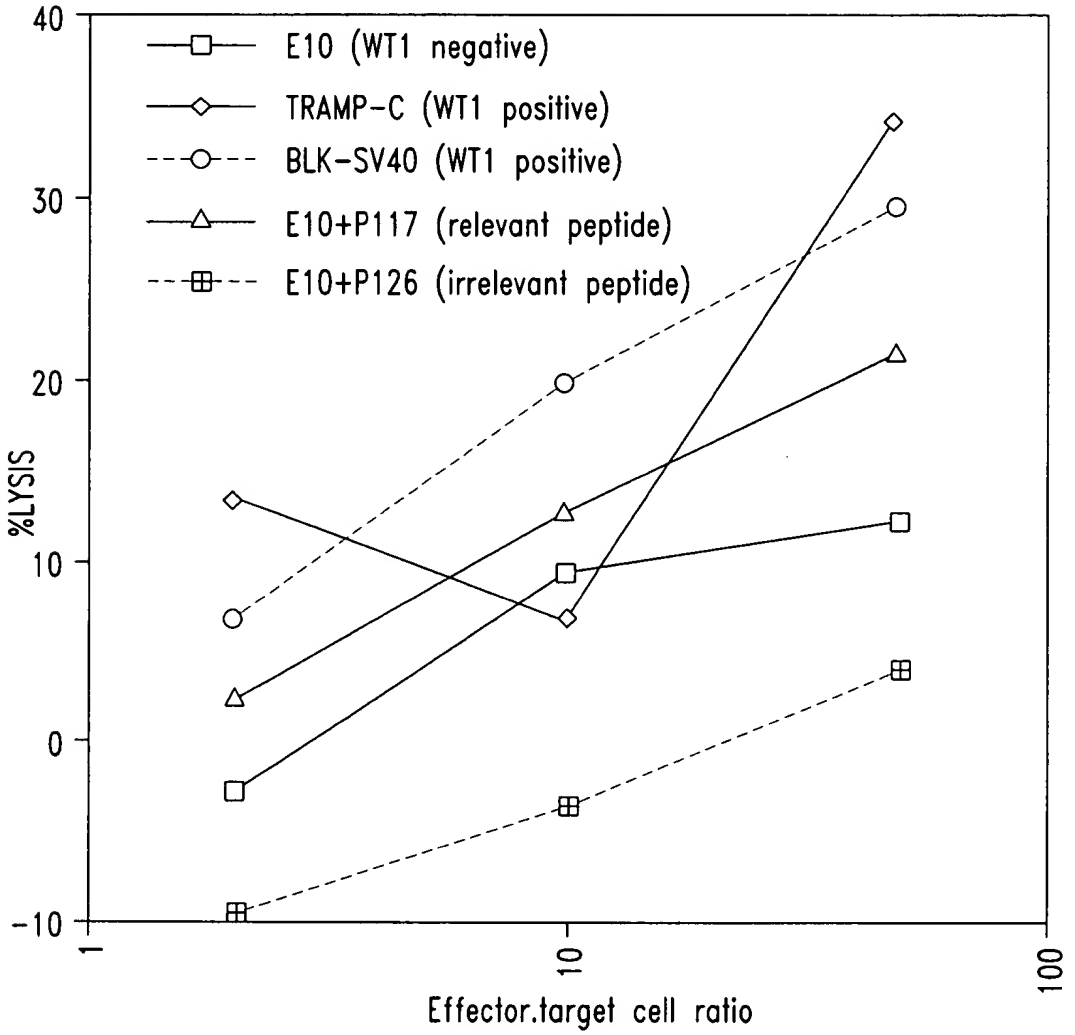
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
E10+P117 (relevant peptide)
E10+P126 (irrelevant peptide)
%LYSIS
40
30
20
10
0
-10
1
10
100
Effector.target cell ratio

Fig. 10C

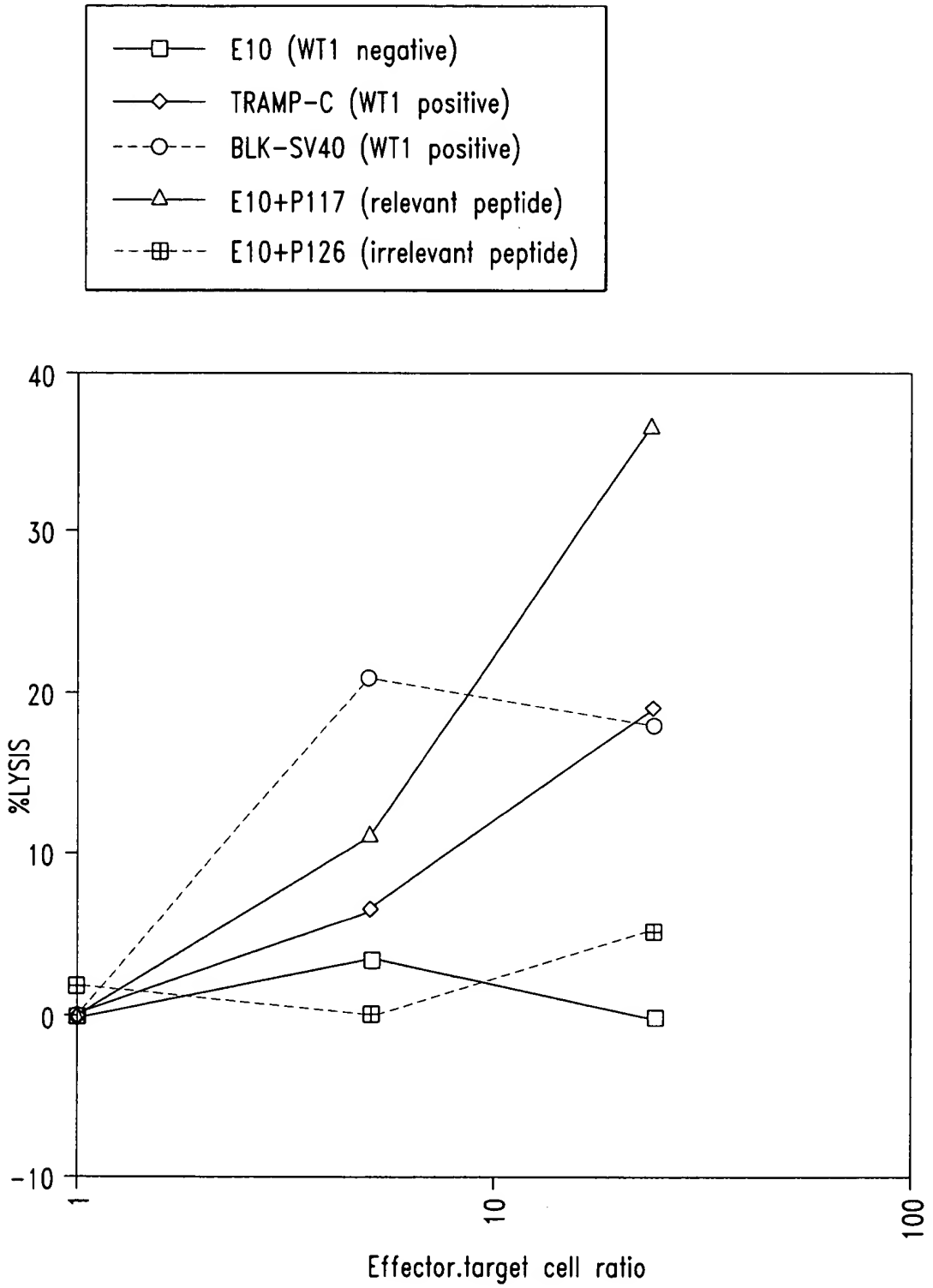
E10 (WT1 negative)
TRAMP-C (WT1 positive)
BLK-SV40 (WT1 positive)
E10+P117 (relevant peptide)
E10+P126 (irrelevant peptide)
40
30
20
10
0
-10
%LYSIS
1
10
100
Effector.target cell ratio

Fig. 10D

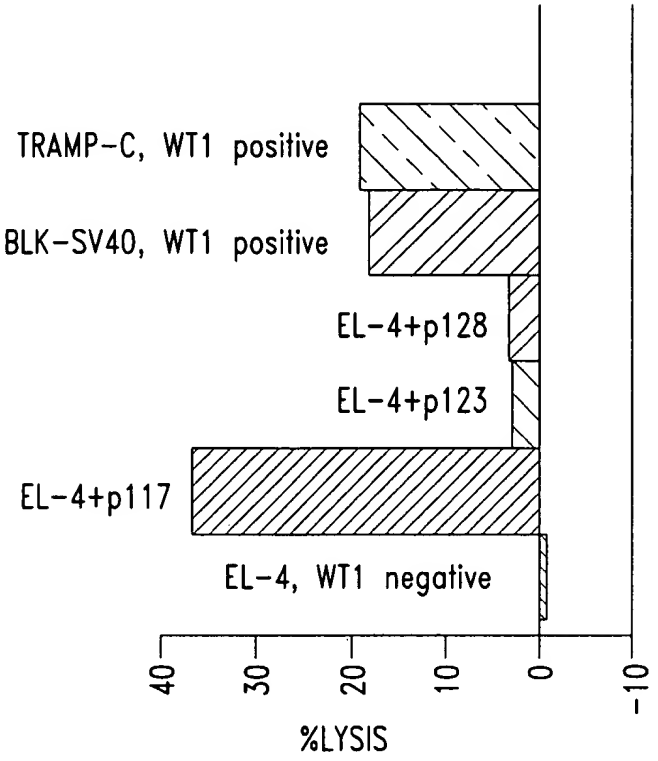

Fig. 11B

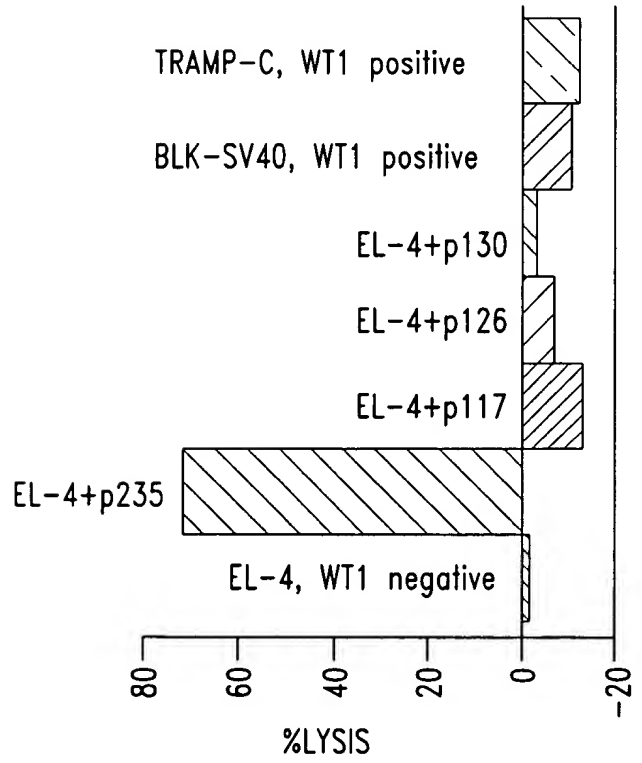

Fig. 11A

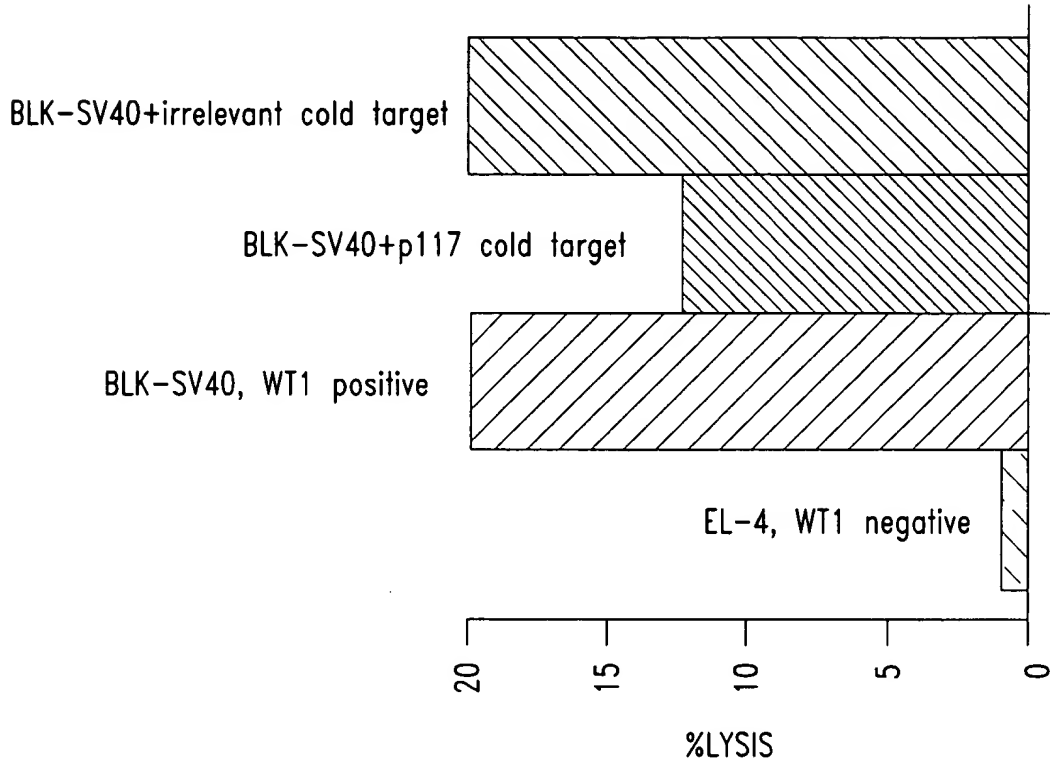

Fig. 12B

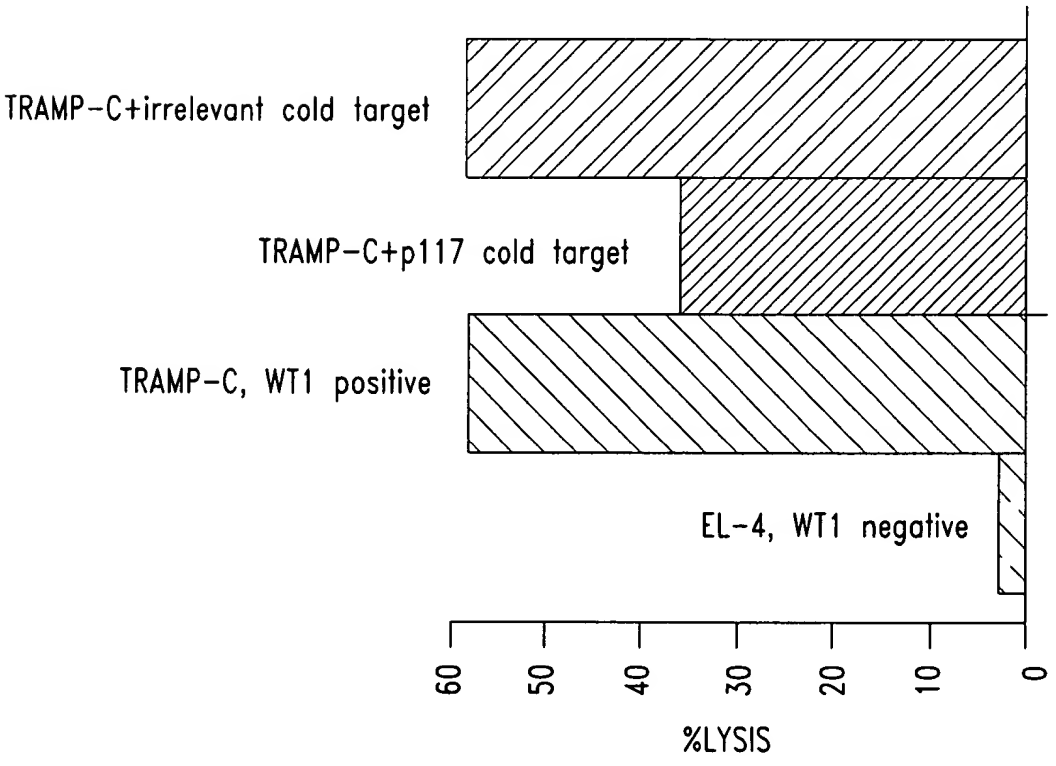

Fig. 12A

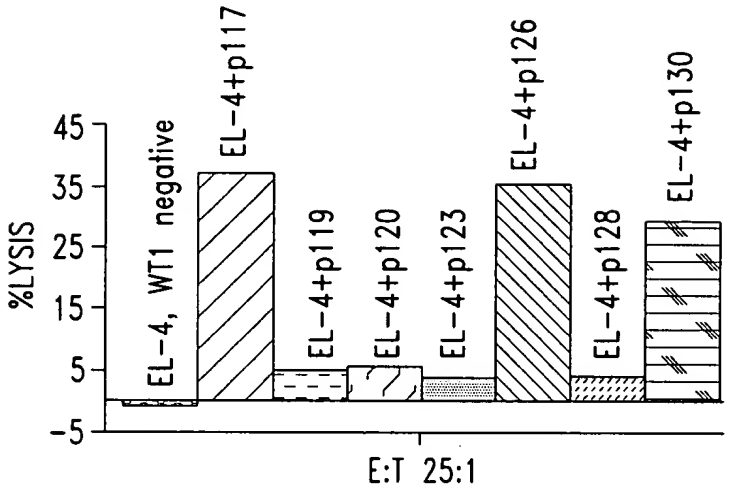

Fig. 13A

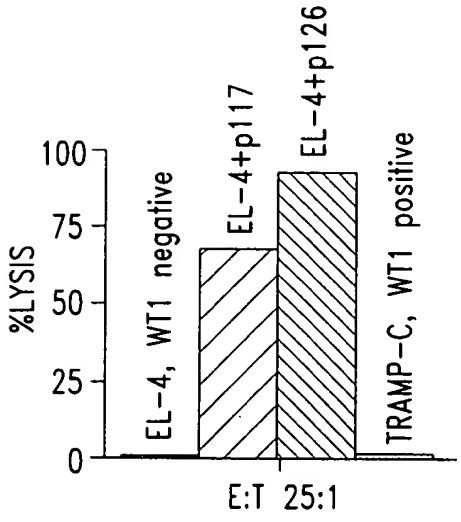

Fig. 13B

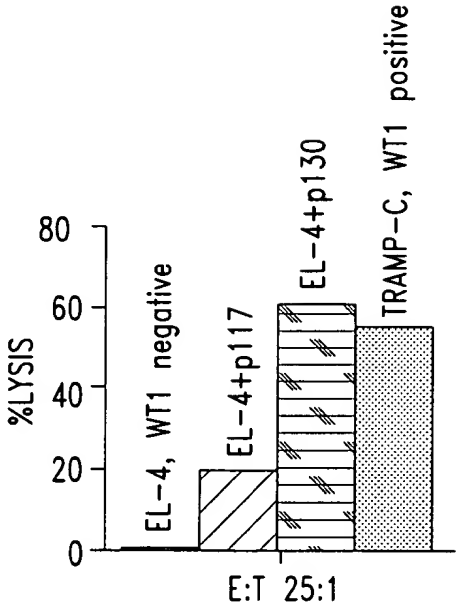

Fig. 13C

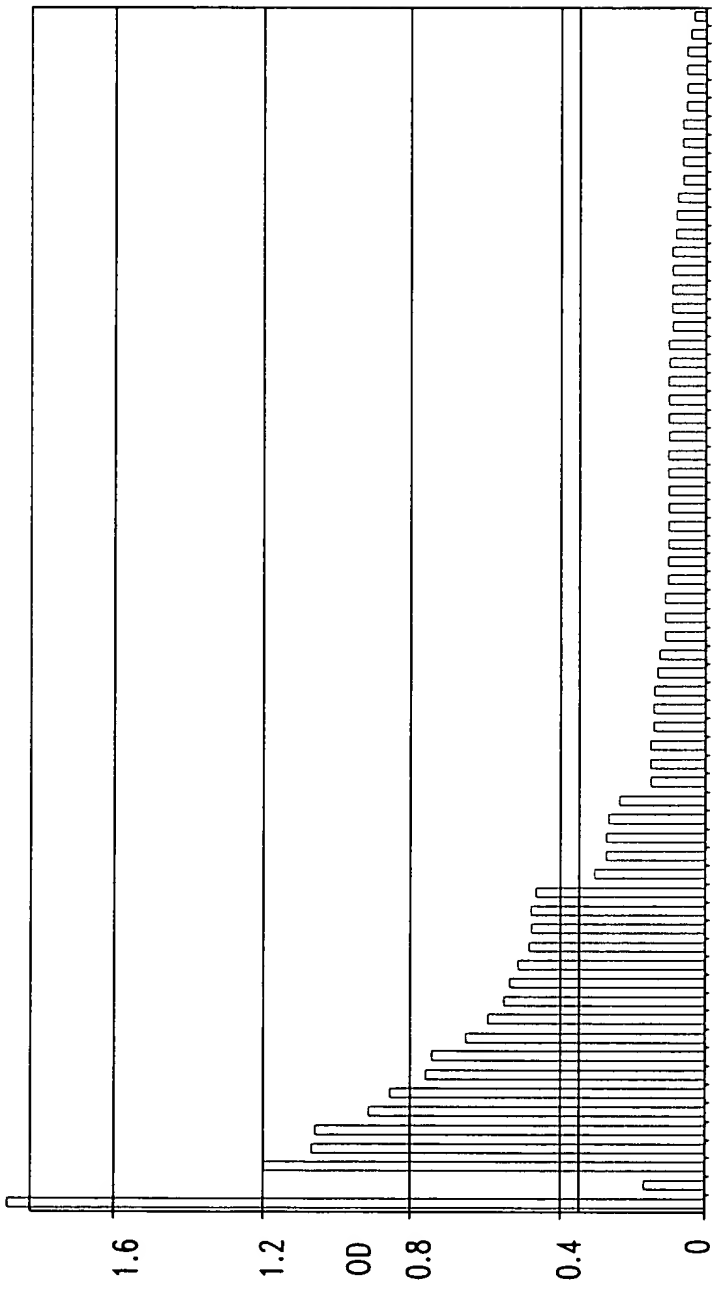

Fig. 14

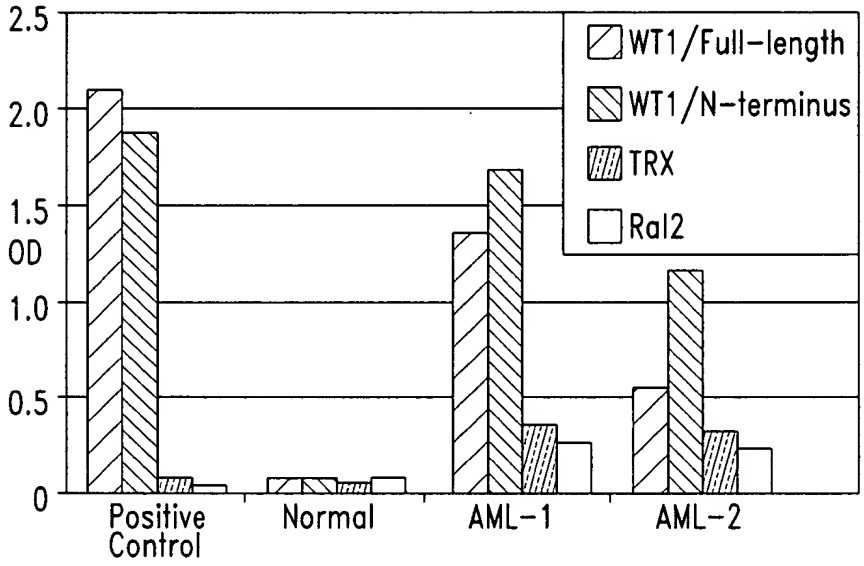
2.5
2.0
1.5
OD
1.0
0.5
0
WT1/Full-length
WT1/N-terminus
TRX
Ral2
Positive Control
Normal
AML-1
AML-2

Fig. 15

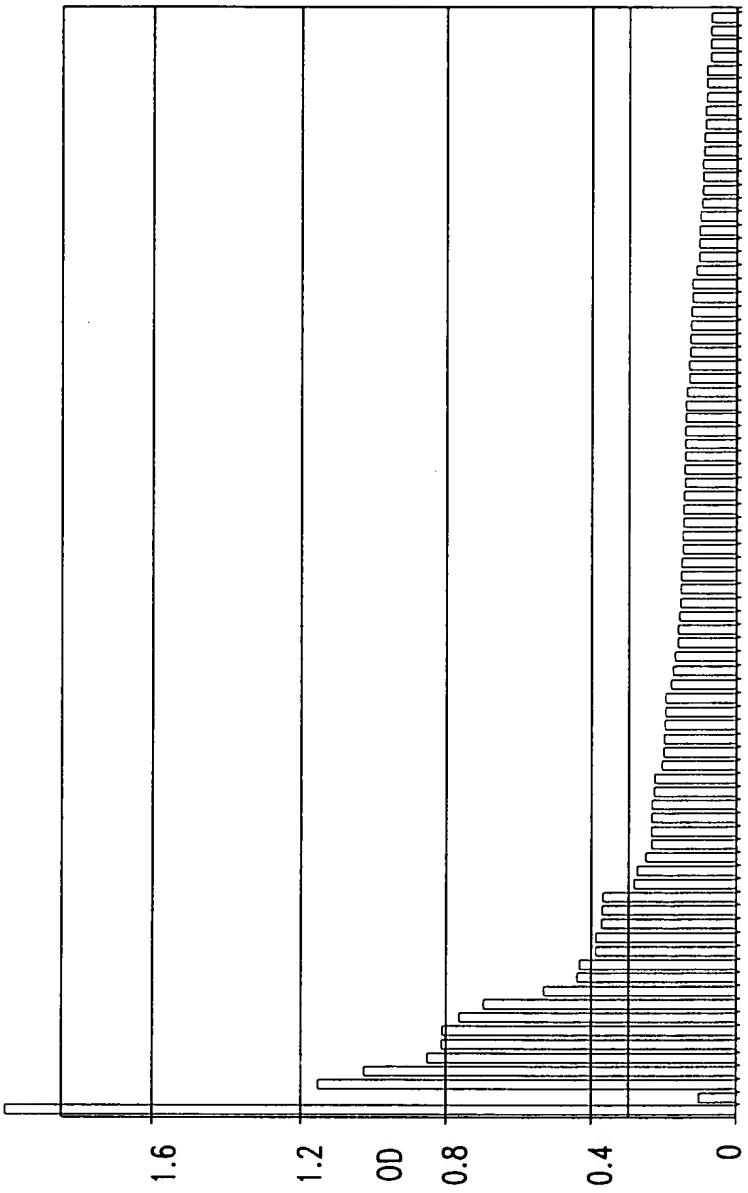

Fig. 16

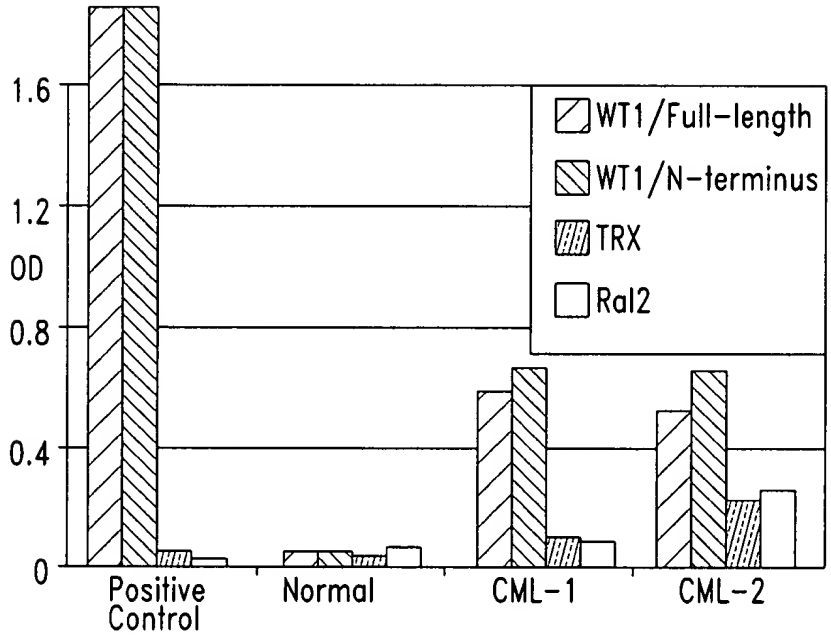
1.6
1.2
OD
0.8
0.4
0
WT1/Full-length
WT1/N-terminus
TRX
Ral2
Positive Control
Normal
CML-1
CML-2

Fig. 17

Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

NAME	Recombinant Protein	WT1 Amino Acid Position	Molecular Weight
WT1/full-length	Ral2-WT1 full length fusion protein	aa 1-449	85kDa
WT1/N-terminus	TRX-WT1 N-terminus fusion protein	aa 1-249	60kDa
WT1/C-terminus	WT1 C-terminus protein	aa 267-449	50kDa

Fig. 18

WT1 Specific Serum Antibodies in Patients with AML and CML

	WT1/full-length	WT1/N-terminus	WT1/C-terminus
Normal Individials (n=96)	2/96 (2%)	1/96 (1%)	1/96 (1%)
AML Patients (n=63)	14/63 (22%)	16/63 (25%)	2/63 (3%)
CML Patients (n=81)	15/81 (19%)	12/81 (15%)	3/81 (3%)

Fig. 19